ISSN 0003-6838, Applied Biochemistry and Microbiology, 2016, Vol. 52, No. 6, pp. 593–601. © Pleiades Publishing, Inc., 2016.

Efficient Inhibition of Some Multi-Drug Resistant Pathogenic Bacteria by Bioactive Metabolites from *Bacillus amyloliquefaciens* S_5I_4 Isolated from Archaeological Soil in Egypt[1]

F. M. Reda*, S. A. Shafi, and M. Ismail

Botany and Microbiology Department, Faculty of Science, Zagazig University, Zagazig, Egypt

**e-mail: afm67@yahoo.com, fifi.reda133@yahoo.com*

Received February 19, 2016

Abstract– Sixty-eight bacterial cultures were isolated from 5 archaeological soils in Egypt. It is necessary to characterize bacteria from ancient temples to develop protection programs for such archaeological places. Purified bacterial cultures were then tested for their capability to inhibit some multi-drug resistant (**MDR**) pathogenic bacteria including *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus, Escherichia coli* and *Klebsiella pneumoniae*. Among the most active 10 antibacterial isolates, only one isolate designated as S_5I_4 was selected, characterized and identified as belonging to *Bacillus amyloliquefaciens*. The strain identification was confirmed by amplification of its 16S rRNA gene. The partial nucleotide sequence of the amplified 16S rRNA gene of the tested strain was submitted in GenBank with accession number AB813716. The physical and nutritional parameters were optimized to improve the production of antimicrobial agents by the *B. amyloliquefaciens* S_5I_4. The maximum antagonistic effect of this strain against the tested MDR pathogenic bacteria was achieved in presence of 1% galactose and 0.5% yeast extract at 37°C and pH 7.0 after 48 h incubation. The antibacterial compounds of *B. amyloliquefaciens* S_5I_4 were extracted, purified and characterized using spectroscopic analysis (IR, UV, proton NMR and MS). The compound having inhibitory activity was identified as butanedioic acid, octadecyl,1(1carboxy1methylethyl) 4octyl ester.

Keywords: Bacillus amyloliquefaciens, antagonistic effect, bioactive agents, multi-drug resistant pathogenic bacteria

DOI: 10.1134/S0003683816060144

Resistance to antibiotics has become a serious problem; therefore efforts have been exerted to identify novel compounds with antibacterial activity. These compounds are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections [1]. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. Consequently, there is a need to continue research to find out other alternative bacterial metabolites or novel natural agents with board activity against multi-drug resistant (**MDR**) pathogenic bacteria. One of the methods to fight the antibiotics resistance is by discovering new antimicrobial agents [2]. The need for novel substances to combat increased antibiotic resistance in pathogenic bacteria has stimulated the exploration of other than the traditional sources, such as terrestrial actinomycetes or fungi [3]. Bioactive secondary metabolites like glycosides, saponins, tannins, alkaloids, sterols and terpenes are believed to play an essential role in microbial interactions by mediating antagonistic activity and intercellular communication [4].

Production of antibacterial compounds seems to be a general phenomenon for most bacteria, an admirable array of microbial defense systems are produced including broad–spectrum classical antibiotics, metabolic by-products such as organic acids and lytic agents. In addition, several types of V protein exotoxins and bacteriocins, which are biologically active peptide moieties with bactericidal mode of action, were described [5].

Compounds from *Bacillus amyloliquefaciens* strain, have attracted a considerable interest and such substances as terpenoids and terpene alcohols were studied in details. Alkylation and hydroxylation of the terpenoides, phenols and fatty acids increase their antimicrobial activity [6]. Inoue et al. [7] studied the antibacterial effects of 3 terpene alcohols against *Staphylococcus aureus*. They suggested that the terpene alcohols, namely, farnesol, nerolidol and plaunotol might damage cell membranes which were one of the major modes of action of these compounds. Zengin

[1] The article is published in the original.

Table 1. Sources of tested MDR pathogenic strains

Tested MDR pathogenic strain	Source	Accession number
S. aureus	Urine	KF771028
L. monocytogenes	Double cream cheese	LMG10470
B. cereus	Minced meat	JX455159
E. coli	Urine	KF771030
K. pneumoniae	Sputum	KF771031

and Baysal [8] investigated the antibacterial activity and antioxidant effect of α-terpineol, linalool, eucalyptol and α-pinene obtained from essential oils; these substances inhibited the growth of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *S. aureus*. The antimicrobial activity of terpenoids was related to their functional groups, and the hydroxyl group of the phenolic terpenoids and the presence of delocalized electrons were revealed as important elements for their antimicrobial action [9].

Taking into account that isolation and characterization of bacteria from ancient temples is important for the development of protection archaeological programs the aim of the study was to (i) isolate and identify the antimicrobial agents produced by bacteria from different ancient archaeological soils in Egypt, (ii) optimize the production of antimicrobial agents against MDR pathogenic bacteria (*Staphylococcus aureus* KF771028, *Listeria monocytogenes* LMG10470, *Bacillus cereus* JX455159, *Escherichia coli* KF771030 and *Klebsiella pneumonia* KF771031) and (iii) characterize and identify the bioactive compounds produced by selected strain.

MATERIALS AND METHODS

Soil samples collection. Five soil samples were collected from different archaeological regions of Egypt according to the procedure described by Johonson et al. [10]. The archaeological regions selected for the study were Fakous, San El Hager and Tell Basta (Sharkia governorate), Giza pyramids (Giza governorate) and Idfou Temple (Aswan governorate).

Microbiological analysis. *Isolation and purification of the bacterial isolates.* Bacterial cultures were isolated from the collected soil samples by using standard dilution plate procedure [11]. Representatives of single and pure colonies were picked up by sterile metal loop and streaked on to brain heart infusion (BHI) agar medium (Oxoid Ltd., UK). Slope cultures were then made and stored at 4°C in a refrigerator throughout the experiments.

Identification of bacteria. The pure bacterial cultures were identified according to standard clinical laboratory methods reported and recommended by Bergey's Manual of Systematic Bacteriology [12].

Molecular identification of bacteria using 16S rRNA analysis. Total DNA was extracted from the selected bacterial isolate according to Sambrook and Russel [13]. The gene encoding 16S rRNA was amplified by PCR with universal primers (forward primer [F27] 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer [R1492] 5'-GGTTACCTTGTTACGACTT-3') [14]. The amplification conditions were as follows: 94°C for 10 min and 35 cycles of denaturation at 95°C for 30 s, annealing-extension at 56°C for 1 min, 72°C for 1 min and an extension at 72°C for 10 min. Presence and yield of specific PCR products (16S rRNA gene) were monitored by running 1% agarose gels. Then the PCR product was cleaned up by using GeneJET™ PCR purification kit (Thermo Fisher Scientific, USA).

Amplified DNA fragment was partially sequenced at GATC Biotech AG (Germany) using ABI 3730xl DNA sequencer using forward primer F27. A sequence of 16S rRNA gene of the selected strain was deposited at NCBI web server (www.ncbi.nlm.nih.gov). Sequence analysis and comparison to published sequences were made using the BLAST program (http://www.ncbi.nlm.nih.gov/blast) [15]. Multiple sequence alignment and molecular phylogeny were performed using BioEdit software. The phylogenetic tree was displayed using TreeView program.

Antimicrobial activity assay of bacterial isolates. The bacterial isolates were screened for their capability to inhibit some MDR pathogenic bacterial strains including *S. aureus* KF771028, *L. monocytogenes* LMG10470, *B. cereus* JX455159, *E. coli* KF771030 and *Klebsiella pneumoniae* KF771031 (Table 1), kindly provided by Bacteriology Laboratory, Faculty of Science, Zagazig University, Egypt. One mL (10^5 CFU) of 24 h age culture of bacterial isolates were inoculated in 100 mL nutrient broth medium containing (g/L): peptone – 5.0; NaCl – 5.0; beef extract – 2.0; yeast extract – 1.0 (pH 7 ± 0.2) and incubated at 37°C for 24 h. Cultures were centrifuged at 6000 × g for 20 min at 4°C. The cell-free supernatants (CFSs) were sterilized by filtration through a 0.22 μm Millipore filter (USA).

The antimicrobial spectra of the CFSs of the tested isolates were determined using the disc diffusion method according to Tagg and Mc-Given [16]. The MDR pathogenic bacterial strains were cultured onto nutrient agar (Oxoid Ltd., UK) for 24 h at 37°C, and used to prepare cell suspensions in 9 mL normal saline solution. Twenty mL of nutrient agar cooled to 45°C was mixed with 100 μL of the pathogenic strains suspension, pooled in Petri dishes and incubated aerobically for 2 h at 37°C. Sterilized filter paper discs (diameter of 6 mm) saturated with 100 μL of CFSs were placed on surface of seeded nutrient agar plates and

incubated at 37°C for 24 h. Inhibition zones were determined and measured in mm.

Optimization of culture conditions for growth and antibacterial activity of B. amyloliquefaciens S_5I_4 strain. Nutrient broth medium was subjected to stepwise optimization to get maximum antibacterial activity of *B. amyloliquefaciens S_5I_4*. Different carbon (mannitol, lactose, galactose, maltose, starch, glucose and sucrose) and nitrogen ($NaNO_3$, KNO_3, $NaNO_2$, NH_4NO_3, tryptone, L-Asp, yeast extract, casein and peptone) sources were separately added at varied concentrations. The incubation was performed at pH values from 3.0 to 10.0) and temperatures from 20 to 40°C for different incubation periods (24–72 h). The antibacterial agent production was carried out in 250 mL Erlenmeyer flask containing 50 mL of sterile medium. Flasks were sterilized and inoculated with 1 mL of overnight culture of the selected strain and then incubated at 37°C for 48 h. The antibacterial efficacy of the *B. amyloliquefaciens* S_5I_4 by-product was tested using cell free filtrates **(CFS)** against MDR pathogenic bacteria. Well diffusion inhibition assay was conducted as described by Cintas et al. [17]. The diameters of inhibition zones (mm) were measured.

Extraction, purification and identification of bioactive compounds obtained from *B. amyloliquefaciens* S_5I_4 strain. *Extraction.* Three liters from *B. amyloliquefaciens* S_5I_4 (48 h age) culture growing in optimized production medium at 37°C and pH 7.0 were prepared. The CFS was exposed overnight separately to 10 organic solvents 1 : 1 (vol/vol); absolute ethyl alcohol, ethyl acetate, acetone, methanol, pentanol, methylene chloride, cyclohexane, chloroform, diethyl ether and petroleum ether. The mixture was shaken and separated using a separating funnel thrice. The organic phase was collected and evaporated under reduced pressure by using rotary evaporator. The evaporation was continued until amount of 6 mL [18]. The extracts were assayed against the MDR pathogens as described above.

Thin layer chromatography (TLC). The ethyl acetate extract was analyzed by TLC using chloroform : acetic acid : water (2 : 1 : 1, vol : vol : vol) as the developing solvent system [19]. TLC plates using silica gel 60 F_{254} (Merck, Germany) were pre-coated on thin glass sheets (size: 5 × 20 cm, layer thickness: 0.2 mm; Merck, Germany). TLC plates were air dried and examined under UV apparatus (Spectroline, Model: CX-21/F, long wave UV_{365} and short wave UV_{254}, USA). The R_f value for each spot (blue and green) was determined to test antibacterial substances activity. The antibacterial test was carried out as previously mentioned [17] and the % of inhibition was calculated.

Spectroscopic and the elemental analysis of the purified antibacterial substances. IR, UV, proton NMR and MS analyses were carried out in the Microanalytical Center of Faculty of Science of Cairo University (Egypt).

RESULTS AND DISCUSSION

Antibiosis, production of antibiotic compounds and inhibition of the growth of other microorganisms, is the most important mechanism expressed by the antagonistic bacteria [20]. A microbial biological control agent may act against pathogens differently: by weakening or destroying the pathogen, competing for space and nutrients or producing antimicrobial compounds and enzymes that attack the cell components of the pathogens [21]. The new substances produced by certain microorganisms were used as antimicrobial agents instead of synthetic chemicals [22]. In the present study, 68 bacterial colonies were isolated from 5 archaeological soils in Egypt, purified and maintained on nutrient agar slants. The antibacterial efficiency of these isolates was screened for their capability to inhibit some MDR pathogenic bacterial strains (data not shown). It was necessary to isolate and characterize bacteria from ancient temples, to be able to develop protection programs for such archaeological places. According to Poletti et al. [23], microbial contamination was evaluated by enumeration of bacteria, actinomycetes and fungi present on ancient wall paintings of medieval churches of the Campania region (Italy). In this connection, 46 *Streptomyces* strains were isolated from paintings and stone surfaces from Tell Basta and Tanis Tombs (80 km south-east Cairo, Egypt). Eight of these strains were selected to determine their sensitivity against 13 antibiotics [24]. De-Chao et al. [25] isolated and characterized bacterial strains from ancient (Neogene) permafrost sediment that was permanently frozen for 3.5 million years. The sampling site was located at Mammoth Mountain in the Aldan river valley in Central Yakutia in Eastern Siberia (Russia). These species were characterized with regard to ability to grow at varied temperatures and on different media, in the presence of NaCl, antibiotics and heavy metals and were tested for sensitivity to antibiotics.

In this study, 10 bacterial isolates (15%) exhibited the highest antibacterial activities were selected for further screening against tested MDR pathogenic bacteria (Table 2). The results revealed that S_5I_4 isolate had broad spectrum effect against most tested pathogenic bacteria. It was observed that *S. aureus* strain was the more sensitive organism, while *E. coli* showed the lowest sensitivity. These results coupled with the findings previously published by Zhao et al. [26]. The resistance of bacterial isolates to antibiotics may be due to thickening of cell wall and deposition of outer membrane protein and modification of specific site (s) receptors [27].

Table 2. Screening activities of selected bacterial isolates against certain MDR pathogenic bacteria*

Selected isolate	Tested MDR pathogenic bacteria Inhibition diameter, mm				
	S. aureus KF771028	*L. monocytogenes* LMG10470	*B. cereus* JX455159	*E. coli* KF771030	*K. pneumoniae* KF771031
S_1F_7	10.00 ± 0.50^{fg}	9.00 ± 0.50^{d}	$13.00^{ef} \pm 0.50$	7.00 ± 1.00^{g}	10.00 ± 0.50^{de}
S_1F_8	11.00 ± 0.50^{ef}	11.00 ± 1.00^{c}	14.00 ± 1.00^{de}	10.00 ± 0.50^{f}	12.00 ± 0.50^{bc}
S_2H_9	13.00 ± 0.50^{cd}	9.00 ± 0.50^{d}	10.00 ± 0.50^{g}	11.00 ± 1.00^{ef}	9.00 ± 1.00^{e}
S_2H_{10}	10.00 ± 0.50^{fg}	11.00 ± 1.00^{c}	15.00 ± 1.00^{cd}	12.00 ± 0.50^{de}	11.00 ± 0.50^{cd}
S_3T_5	15.00 ± 0.87^{b}	17.00 ± 0.87^{a}	17.00 ± 0.50^{b}	15.00 ± 1.00^{ba}	12.00 ± 1.00^{cd}
S_4GP_4	9.00 ± 1.00^{g}	10.00 ± 0.50^{cd}	11.00 ± 1.26^{f}	13.00 ± 0.50^{cd}	11.00 ± 1.00^{cd}
S_4GP_6	12.00 ± 1.26^{de}	11.00 ± 1.00^{c}	13.00 ± 1.32^{ef}	14.00 ± 1.32^{bc}	11.00 ± 1.00^{cd}
S_5I_4	23.00 ± 0.58^{a}	17.00 ± 0.50^{a}	20.00 ± 0.50^{a}	14.00 ± 0.50^{a}	16.00 ± 1.00^{a}
S_5I_5	14.00 ± 1.00^{bc}	16.00 ± 1.00^{a}	15.00 ± 0.50^{cd}	11.00 ± 1.00^{ef}	13.00 ± 1.00^{b}
S_5I_8	12.00 ± 0.50^{de}	14.00 ± 1.00^{b}	16.00 ± 1.32^{bc}	7.00 ± 1.00^{g}	10.00 ± 0.50^{de}

* The different letters in each row mean significant effect. S_1 – soil 1; S_2 – soil 2; S_3 – soil 3; S_4 – soil 4; S_5 – soil 5. F – Fakous; H – San El-Hagar; T – Tell Basta; GP – Giza Pyramids; I – Idfou Temple

S_5I_4 isolate was identified according to Bergey's Manual for Systematic bacteriology [14] as *Bacillus amyloliquefaciens.* The identification of *B. amyloliquefaciens* S_5I_4 was molecularly confirmed by investigation of 16S rRNA analysis. Sequence data were submitted to GenBank at NCBI web site (www.ncbi.nlm.nih.gov) with accession number AB813716. BLAST program (www.ncbi.nlm.gov/blast) for phylogenetic analysis was used to assess the similarities of obtained 16S rDNA gene sequence (Fig. 1).

The biosynthesis of secondary metabolites by *Bacillus* spp. is controlled by several factors as medium

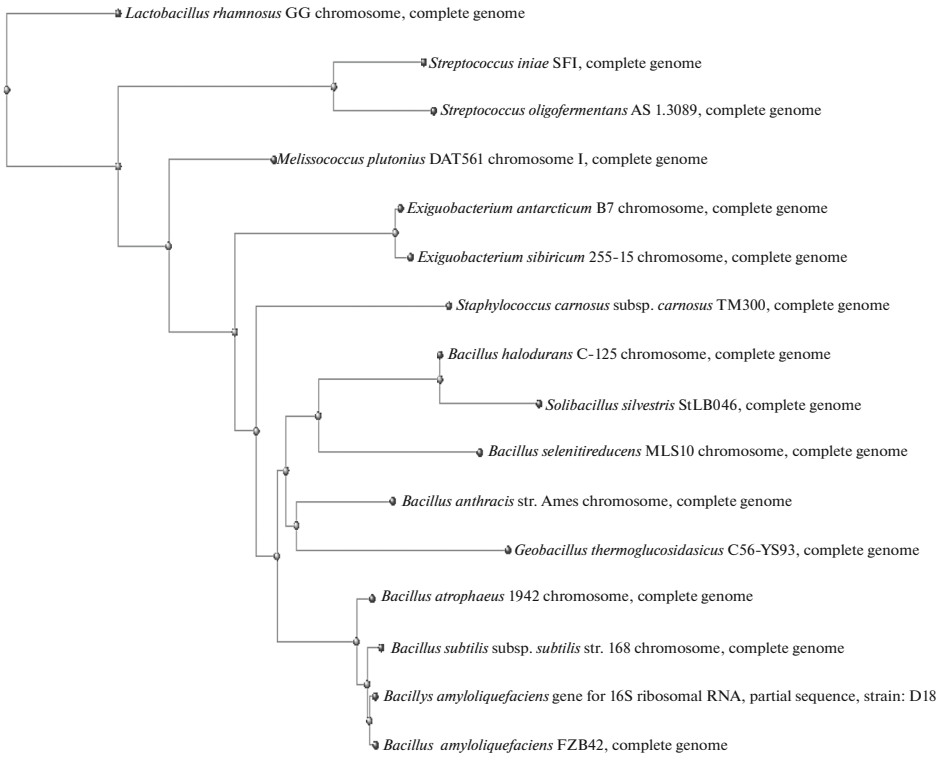

Fig. 1. Phylogenic tree showing location *B. amyloliquefaciens* S_5I_4.

Table 3. Antibacterial activities of the extracted purified compounds produced by *B. amyloliquefaciens* S_5I_4

Tested MDR pathogenic bacteria	Diameter of inhibition zone, mm*		
	compound 1	compound 2	compound 3
S. aureus KF771028	27 ± 3^{b}	30 ± 2^{ab}	33 ± 2^{a}
L. monocytogenes LMG10470	22 ± 2^{b}	24 ± 2^{ab}	26 ± 1^{a}
B. cereus JX455159	25 ± 2^{a}	26 ± 2^{a}	29 ± 3^{a}
E. coli KF771030	19 ± 2^{b}	20 ± 2^{ab}	24 ± 3^{a}
K. pneumoniae KF771031	21 ± 3^{b}	22 ± 2^{b}	27 ± 2^{a}

* The different letters in each row mean significant effect.

composition and cultivation conditions involved a highly complex regulation [28]. Therefore, optimization of conditions is necessary for biosynthesis of the inhibitory compounds from microorganisms. The modified nutrient broth medium was used as a basal medium. This medium was subjected to different conditions as supplementation with and/or replacement of nutrients to evaluate the growth and production of inhibitory compound by S_5I_4 isolate. Incubation period, temperature, pH value, carbon and nitrogen sources were changed to find optimal cultural conditions for the activities of *B. amyloliquefaciens* S_5I_4 against MDR pathogenic bacteria (Fig. 2). For the tested bacterium, the maximum antibacterial activity against most MDR pathogens occurred in medium supplemented with 1% galactose and 0.5% yeast extract (pH 7.0) at 37°C after 48 h incubation. These results are in agreement with those of Hung-Yun et al. [29] who reported that the iturin A production was affected to different levels by application of pH control between 4.0 and 9.0. The optimal value of the pH of the medium for iturin A production by *B. amyloliquefaciens* B128 was found to be 6.64. In this connection, Prasad et al. [30] revealed that the highest yield of exopolysaccharides from *B. amyloliquefaciens* BPRGS strain was found when yeast extract was supplied as the nitrogen source.

The antibacterial compounds produced by *B. amyloliquefaciens* S_5I_4 were extracted, purified and identified using IR, UV, NMR and MS analysis. The bacteria were grown in optimized and modified nutrient broth medium adjusted at pH 7.0 for 48 h at 37°C. CFS was collected and treated with different organic solvents; the organic phases were collected and tested against MDR pathogenic bacteria. It was found that the ethyl acetate was the most efficient solvent for extraction of the active antibacterial substances produced by *B. amyloliquefaciens* S_5I_4. TLC analysis of the extract indicated that 3 major spots of pure bioactive substances were formed at R_f values of 0.169, 0.172 and 0.262 for compounds 1, 2 and 3, respectively (data not shown). The results in Table 3 revealed that compound 3 was the most active fraction against all the tested MDR pathogenic bacterial strains arranged in the following descending order according to the diameter of inhibition zones and the efficiency of purified compound as *S. aureus* > *B. cereus* > *K. pneumonia* > *L. monocytogenes* > *E. coli*. This fact indicated that the tested Gram-positive MDR strains were more sensitive than Gram-negative bacteria. These results are in agreement with those published by Sutyak et al. [31] reported that *B. amyloliquefaciens* produced subtilisin which possesses antimicrobial activity against a variety of pathogenic organisms, including *Gardnerella vaginalis*, *L. monocytogenes*, and *Streptococcus spp* (Group B *Streptococcus*).

Different analysis techniques were used for characterization of the most antibacterial compound 3. The IR spectrum of the purified compound 3 was carried out in the range of 4000–400 cm^{-1} and the most effective bands were revealed at 3463, 1638 and 548 cm^{-1} which correspond to ν(OH), ν(C=O) and ν(CH) groups, respectively [32]. UV–Vis spectral data of the compound 3 was recorded from 200 to 800 nm as shown in Fig. 3. Band at 270 nm may be assigned to n-π* transitions [33]. Also, a proton NMR of purified compound 3 revealed the signals at 1.079 for (allylic methyl), 1.65 for (allylic methyl), 3.33, 3.324 and 3.236 for 3 (methoxy groups), 3.49 and 3.32 for (2 methoxy groups), 4.46, 4.6, 4.66, 4.664, 4.72, 4.78, 4.79, 4.81, 4.85 and 4.86 for several $(-CH-)_n$ and 12.2 for (OH group). Production of antimicrobial substance(s) by members of *B. amyloliquefaciens* taxon was reported by many investigators. MS of the compound 3 is in a good agreement with the suggested formula $C_{34}H_{64}O_6$, and molecular ion peak (M^+) at $m/z = 568$ (Fig. 4). The compound 3 has no characteristic odor and is soluble in chloroform, ethyl acetate, butanol and petroleum ether.

Based on the IR, NMR, MS and the recommended keys for identification of antibiotics, the suggested name of compound 3 may be butanedioic acid, octadecyl, 1-(1-carboxy1methylethyl) 4-octyl ester and its expected structure is presented in Fig. 5. In this connection, identification of an antibiotic involves

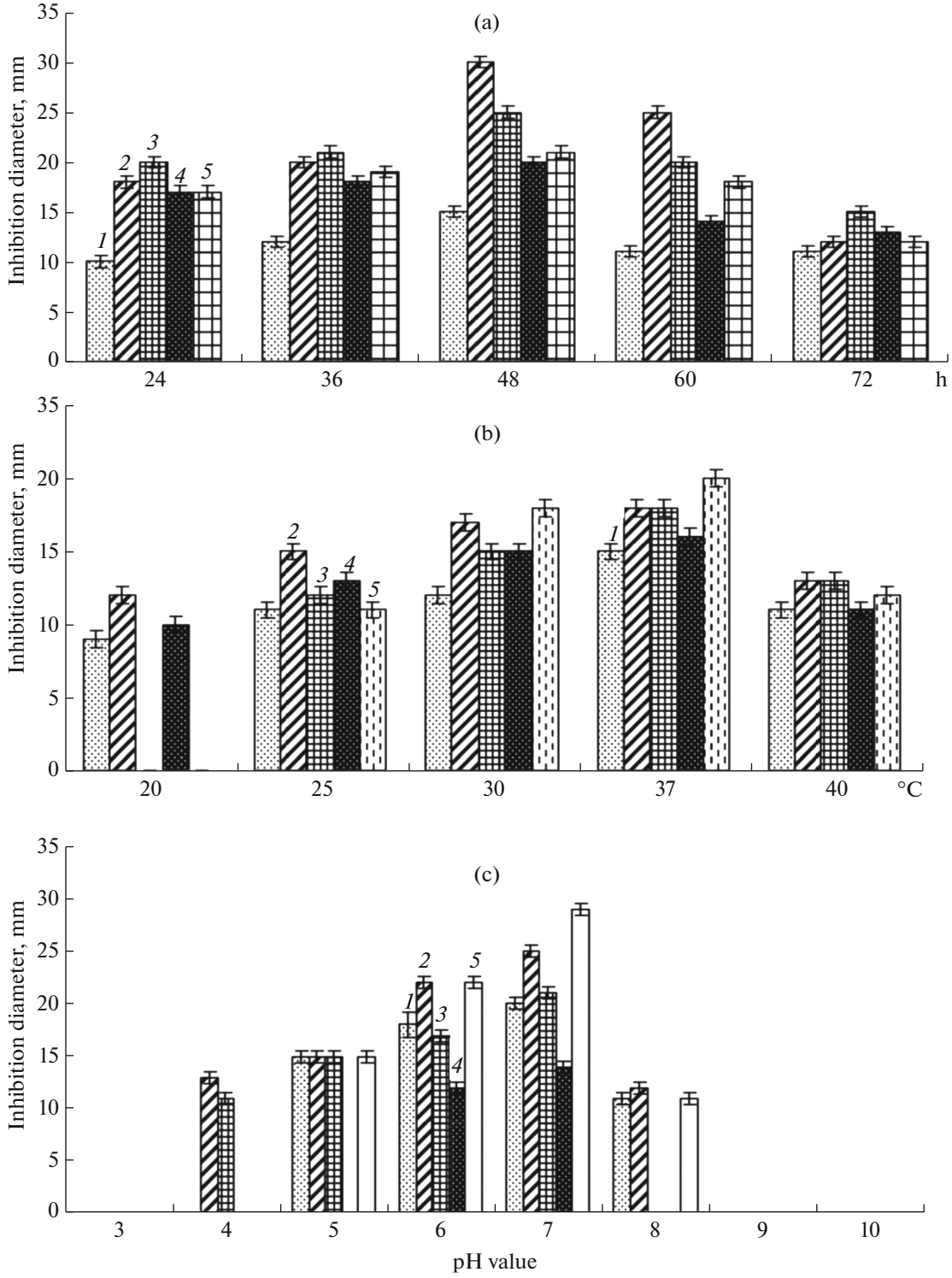

Fig. 2. Optimum cultural conditions for antibacterial production by *B. amyloliquefaciens* S_5I_4 against tested MDR pathogenic bacteria: (a) incubation period; (b) incubation temperatures; (c) pH values; different carbon (d) and nitrogen (e) sources. (*1*) *E. coli* KF771030; (*2*) *K. pneumoniae* KF771031; (*3*) *S. aureus* KF771028; (*4*) *L. monocytogenes* LMG10470; (*5*) *B. cereus* JX455159. In (d): I—maltose; II—glucose; III—galactose; IV—lactose; V—mannitol; VI—starch; VII—sucrose. In (e): I—$NaNO_3$; II—KNO_3; III—NH_4NO_3; IV—$NaNO_2$; V—yeast extract; VI—peptone; VII—casein; VIII—tryptone; IX—L-Asp.

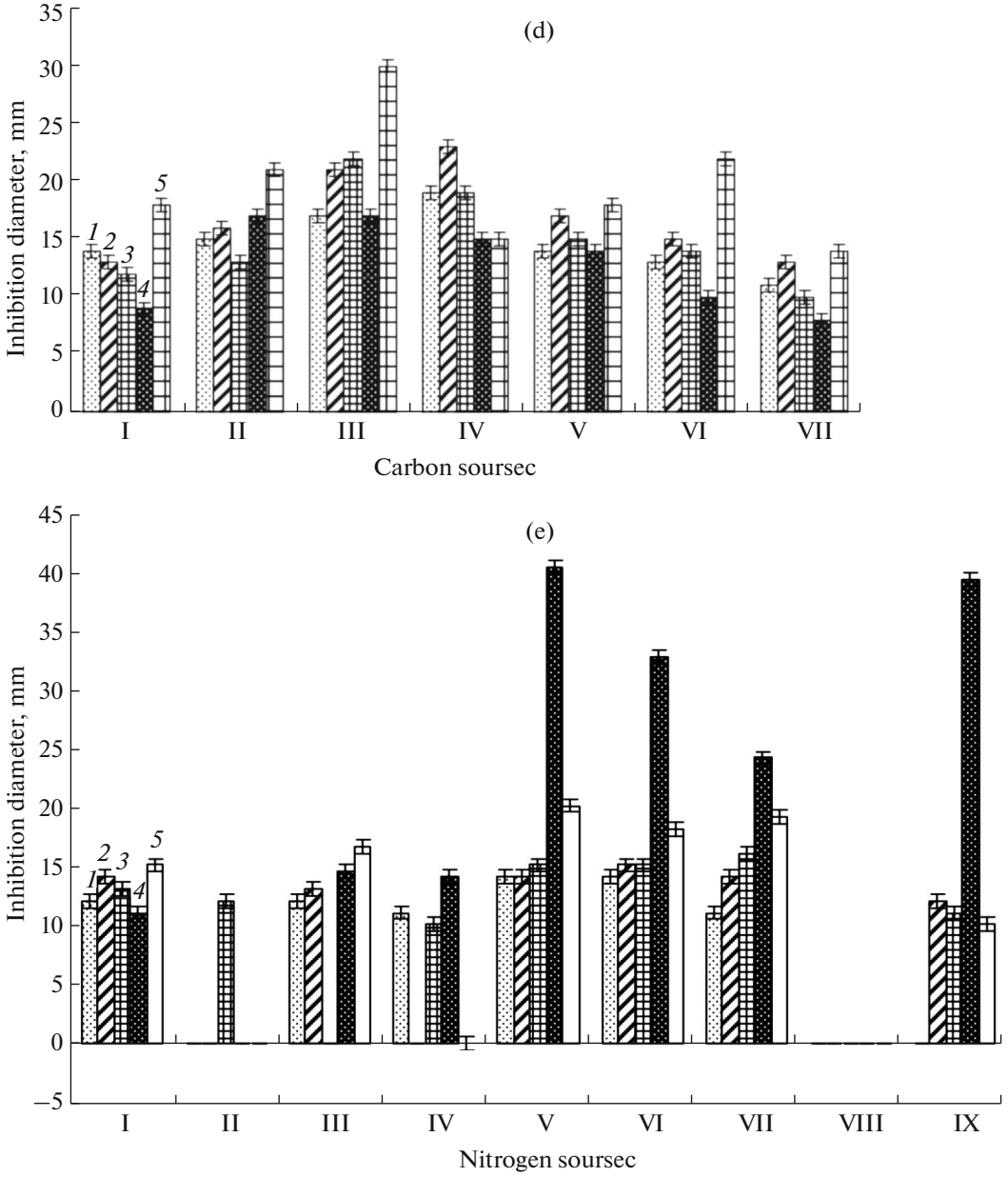

Fig. 2. (Contd.)

two general stages: (1) assignment to a general group and (2) comparison with other member of the group to which it has been assigned in order to see whether or not it is identical with a known compound [34].

In conclusion**,** the extraction, purification and identification of the effective bioactive compound as butanedioic acid, octadecyl, 1-(1-carboxy1methylethyl) 4-octyl ester produced by *B. amyloliquefaciens* S_5I_4 strain (isolated from Idfou Temple, Aswan governorate, Egypt) have been performed. This compound could be used to reduce the growth of MDR pathogenic bacteria including *S. aureus*, *L. monocytogenes*, *B. cereus, E. coli* and *K. pneumonia*.

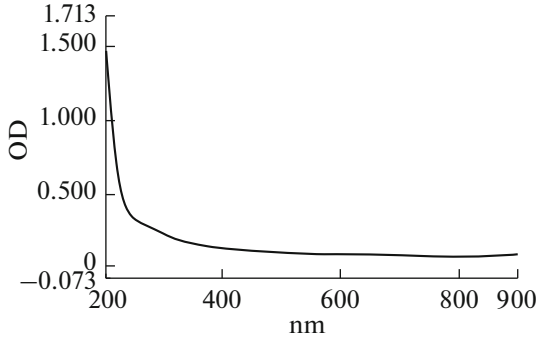

Fig. 3. UV-Vis spectrum of the compound 3 produced by *B. amyloliquefaciens* S_5I_4.

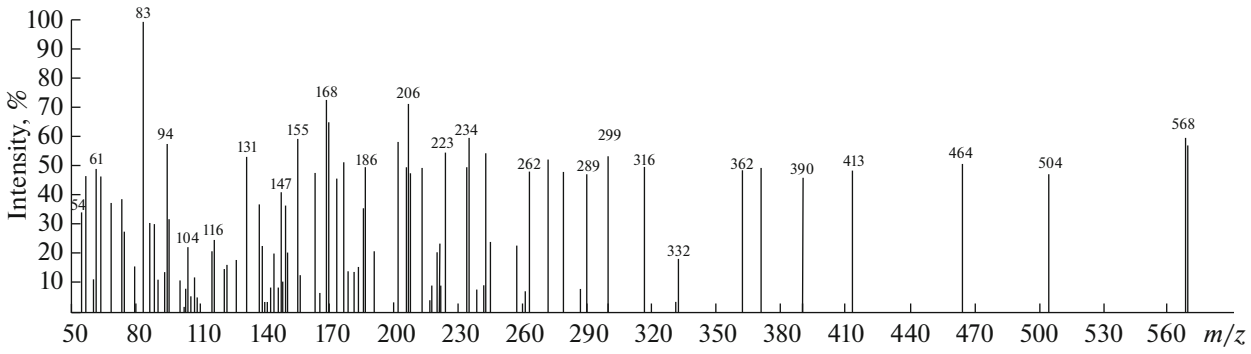

Fig. 4. The mass spectrum of the compound 3 produced by *B. amyloliquefaciens* S_5I_4.

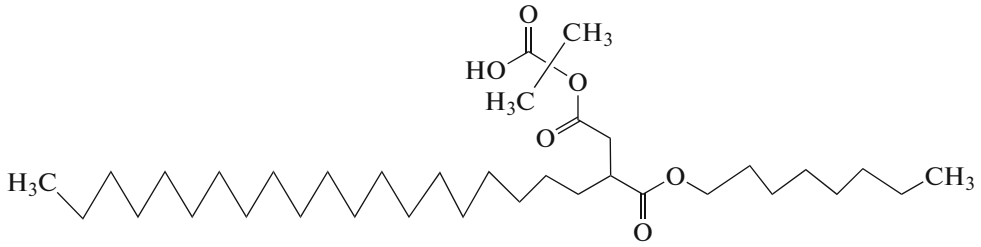

Fig. 5. The structure of compound produced by *B. amyloliquefaciens* S_5I_4.

ACKNOWLEDGMENT

This work was kindly supported by Prof. Dr. A.M. Ateya of Pharmacology Faculty of Pharmacy, Zagazig University (Egypt) and by Ministry of Culture and Archaeology (Egypt).

REFERENCES

1. Westh, H., Zinn, C.S., and Rosdahl, V.T., *Microb. Drug Resist.*, 2004, vol. 10, no. 2, pp. 169–176.
2. Bahvnani, S.M. and Ballow, C.H., *Curr. Opin. Microbiol.*, 2000, vol. 3, no. 5, pp. 528–534.
3. Berdy, J., *J. Antibiot.* (Tokyo), 2005, vol. 58, pp. 1–26.
4. Hibbing, M.E., Fuqua, C., Parsek, M.R., and Peterson, S.B., *Nat. Rev. Microbiol.*, 2010, vol. 8, no. 1, pp. 15–25.
5. Yeaman, M.R. and Yount, N.Y., *Pharmacol. Rev.*, 2003, vol. 55, no. 1, pp. 27–55.
6. Timofeeva, L. and Kleshcheva, N., *J. Appl. Microbiol. Biotech.*, 2011, vol. 89, no. 3, pp. 475–492.
7. Inoue, Y., Shiraishi, A., Hada, T., Hirose, K., Hamashima, H. and Shimada, J., *FEMS Microbiol. Lett.*, 2004, vol. 237, no. 2, pp. 325–331.
8. Zengin, H. and Baysal, A.H., *Molecules*, 2014, vol. 19, pp. 17773–17798; doi 10.3390/molecules191117773
9. Dorman, H.J.D. and Deans, S.G., *J. Appl. Microbiol.*, 2000, vol. 88, no. 2, pp. 308–316.
10. Johnson, L.F., Curl, E.A., Bond, J.H., Fribourg, H.A., *Methods for Studying Soil Microflora–Plant Disease Relationships*, Minneapolis: Burgess Publishing Company, 1960, p. 178.
11. Johnson, L.F., Curl, E.A., Bond, J.H., Fribourg, H.A., *Methods for Studying Soil Microflora–Plant Disease Relationships*, Minneapolis: Burgess Publishing Company, 1959, p. 261.
12. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T., *Bergey's Manual of Determinative Bacteriology*, 9th ed., Baltimore, USA: Williams and Wilkins Co., 1994.
13. Sambrook, J. and Russel, D., *Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory*, New York: Cold Spring Harbor Press, 2001.
14. Turner, S., Pryer, K.M., Miao, V.P.W. and Palmer, D.J., *J. Eukaryotic Microbiol.*, 1999, vol. 46, no. 4, pp. 327–338.
15. Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J., *Nucleic Acids Res.*, 1997, vol. 17, pp. 389–402.
16. Tagg, J.R. and McGiven, A.R., *Appl. Microbiol.*, 1971, vol. 21, no. 5, p. 943.
17. Cintas, L.M., Rodriguez, J.M., Fernandez, M.F., Sletten, K., Nes, I.F., Hernandez, P.E. and Holo, H., *Appl. Environ. Microbiol.*, 1995, vol. 61, no. 7, pp. 2643–2648.
18. Hussein, A.M., Yassin, M.H. and El-Tarabily, K.A., Bull. Fac. Sci. Assiut. Univ., 1998, vol. 27 (I – D), pp. 41–52.
19. Stahl, E, *Thin Layer Chromatography.* 2-nd ed. Berlin-Heidelberg, New York: Springer- Verlage, 1969, p. 857.
20. Intana, W., Yenjit, P., Suwanno, T., Sattasakulchai, S., Suwanno, M., and Chamswarng, C., *Walailak J. Sci. Techn.*, 2008, vol. 5, no. 1, pp. 29–38.

21. Agrios, G.N., *Plant Pathology*, 5-th ed., Oxford: Elsevier Academic Press, 2005.

22. Liu, D., Anderson, N.A., and Kinkel, L.L. *Can. J. Microbiol.*, 1996, vol. 42, no. 5, pp. 487 – 502.

23. Poletti L., Pasquarella C., Pitzurra M. and Savino A., *J. Hosp. Infect.*, 1999, vol. 41, pp. 195–201.

24. Abdel-Haliem, M.E.F., Ali, M.F., Ghaly, M.F. and Sakr, A.A., *J. Cultural Heritage*, 2013, vol. 14, pp. 45–50.

25. De-Chao, Z., Brouchkov, A., Griva, G., Schinner, F. and Margesin, R., *Biology*, 2013, vol. 2, pp. 85-106.

26. Zhao, W.H., Chen, G., Ito, R., Kimura, S. and Hu, Z.Q., *J. Med. Microbiol.*, 2012, vol. 61, no. 2, pp. 246–251.

27. Burt, S., *Int. J. Microbiol.*, 2004, vol. 94, no. 3, pp. 233-253.

28. Akpa, E., Jacque,s P., Wathelet, B., Paquot, M., Fuchs, R., Budzikiewicz, H., and Thonart, P., *App. Biochem. Biotech.*, 2001, vol. 91, no. 1, pp. 551–561.

29. Lin, H.-Y., Rao, Y.K., Wu, W.-S. and Tzeng, Y.-M., *Int. J. Appl. Sci. Eng.*, 2007, vol. 5, no. 2, pp. 123-132.

30. Prasad, V.L, Vandross, A., Toomey, C., Cheung, M., Rho, J., Quinn, S., et al., *Mayo Clin. Proc.*, 2013, vol. 88, no. 8, pp. 790-798.

31. Sutyak, K. E., Wirawan, R. E., Aroutcheva, A. A. and Chikindas, M. L., *J. Appl. Microbiol.*, 2008, vol. 104, no. 4, pp. 1067–1074.

32. Patil, S.A., Naik, V.H., Kulkarni, A.D. and Badami, P.S., *Spectrochim. Acta A.*, 2010, vol. 75, pp. 347-354.

33. Cotton, F.A., Wilkinson, G., Murillo, C.A. and Bochmann, M., *Advanced Inorganic Chemistry*. 6-th ed. New York: Wiley. 1999.

34. Zahner, H. and Mass, W.K., *Biology of Antibiotics*, New York, Berlin: Springer – Verlag, 1972, pp. 114 – 121.